UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

MEMORANDUM

DATE: February 19, 2014
TXR #: 0056896

SUBJECT: **Chlorpyrifos:** Quality Assurance Assessment of the Chlorpyrifos Physiologically Based Pharmacokinetic/Pharmacodynamic Model for Human Health Risk Assessment Applications

PC Code: 059101
Decision No.: N/A
Petition No.: N/A
Risk Assessment Type: N/A
TXR No.: 0056896
MRID No.: 49252601

DP Barcode: D417053
Registration No.:
Regulatory Action:
Case No.: N/A
CAS No.: 2921-88-2
40 CFR: §180.342

FROM: Chester E. Rodriguez, Ph.D., Toxicologist
Health Effects Division (HED)
Office of Pesticide Programs (OPP)

Cecilia Tan, MBA, Ph.D. Physical Research Scientist
National Exposure Research Laboratory (NERL)
Office of Research and Development (ORD)

TO: Joel Wolf, Chemical Risk Manager
Pesticide Re-Evaluation Division (PRD)
Office of Pesticide Programs (OPP)

A quality assurance (QA) assessment was conducted to evaluate the appropriateness of using the multi-route chlorpyrifos physiologically-based pharmacokinetic-pharmacodynamic (PBPK-PD) model to reduce uncertainty across different species, routes of exposure, and lifestages in human health risk assessment. This QA assessment focused on ensuring the model structure and parameter values are accurately reflected and implemented in the computer code, and selected experimental human data can be reasonably simulated by the model. Additionally, this QA assessment also ensured that critical model parameters have been adequately identified and properly applied to simulate human variability in red blood cell (RBC) cholinesterase inhibition, as well as uncertainty in the predictions of RBC cholinesterase inhibition.

Table of Contents

I. Background on the chlorpyrifos PBPK-PD model

PBPK models are the preferred dosimetry tool in human health risk assessment to help reduce uncertainty in the extrapolation across different species, exposures (routes, levels, and duration), and lifestages. The chlorpyrifos PBPK-PD model was originally developed in 2002 by Timchalk and co-workers to track internal dosimetry of chlorpyrifos and its major metabolites [*i.e.*, chlorpyrifos oxon and 3,5,6-trichloro-2-pyridinol (TCPy)], as well as cholinesterase inhibition in various tissues (Timchalk et al., 2002). Since its development, the model has undergone significant refinements as new experimental data has become available, and it has been evaluated to different degrees by the Agency's Scientific Advisory Panel (SAP) in two separate occasions (FIFRA SAP 2011 and 2012). Recently, the model has been expanded to accommodate orally ingested oxon, as well as dermal and inhalation exposures to chlorpyrifos. The chlorpyrifos PBPK-PD model is considered robust because an extensive *in vitro* and *in vivo* database has been used in its development, calibration, and evaluation. Data from two human deliberate dosing studies that include levels of biomarkers in blood and urine and cholinesterase inhibition have been used in model calibration and evaluation. It is important to note, however, that the current model lacks the ability to simulate gestational and lactational exposures for evaluation of potential developmental effects.

II. The chlorpyrifos PBPK-PD model code

The PBPK-PD model computer code was written in the acslX programming language (version 2.5.0.6, Aegis Technologies, Inc., Huntsville, Alabama). Separate code files were provided by the Registrant, Dow AgroSciences (DAS) for the rat and human versions of the model.

III. Verification of model parameters

DAS sponsored Summit Toxicology, LLP to conduct an independent QA assessment of the chlorpyrifos PBPK-PD model for verifying parameter values and their respective sources. In addition, local sensitivity analyses and model simulations were repeated to verify the results published by DAS. EPA conducted a review of this report by randomly checking the values and sources of 10% of the model parameters. Most of the parameter values were correctly implemented in the computational code, and their sources correctly identified. EPA, however, found the following inconsistencies that were not reported in Summit Toxicology's QA report: values for plasma protein binding for chlorpyrifos (FBC) and oxon (FBO) in humans were reported to be 99%, but the reference that was cited (Lowe et al., 2009) had a value of 98% for FBC, and no reported value for FBO. While 99% is a reasonable estimate for both FBC and FBO, these values did not come from the reference that was cited. In fact, because FBO could not be experimentally determined, it was assumed the same value as FBC. This issue which only affected the documentation, not the performance of the model has been addressed in the model files.

IV. Verification of model simulations of human deliberate dosing studies

(a). Nolan Oral Study

The chlorpyrifos PBPK-PD model is considered robust in part because it was calibrated and evaluated using data collected from two controlled, deliberate human dosing studies. Both studies have been evaluated for both scientific and ethical considerations by the EPA Human Studies Review Board. In the first study (Nolan *et al.*, 1984), 6 male volunteers were given an oral dose of 0.5 mg/kg BW in a lactose tablet. The volunteers were 27-50 years old and their body weights (BW) were on average 83.3 kg (±10.3). Blood and urine were analyzed for both chlorpyrifos and its primary metabolite, TCPy. The urinary data collected in the Nolan study suggested that nearly 100% of the dose is excreted as TCPy in urine and these data were used to estimate the TCPy-specific parameters. In addition to chemical concentrations, cholinesterase activity was also measured in both plasma and RBC.

As part of the QA assessment process, the model was independently evaluated for its ability to reasonably reproduce both pharmacokinetic (blood and urinary concentrations) and pharmacodynamic data (cholinesterase activity) from the Nolan 1984 study. In this assessment, model simulations were directly compared against the Nolan data which were obtained from the original Data Evaluation Record (DER). Additionally, this comparison was plotted using a linear scale, rather than a logarithmic scale as used by DAS, since a logarithmic scale tends to visually diminish discrepancies between model predictions and experimental measurements.

The first simulation was the time course of blood concentrations of chlorpyrifos after a single oral exposure to 0.5 mg chlorpyrifos/kg BW. It was found that the model over-predicted blood concentrations of chlorpyrifos by a factor of two (Figure 1A). This range of over-prediction is considered reasonable because of the uncertainty associated with the measurements as these levels were approaching the limit of analytical detection.

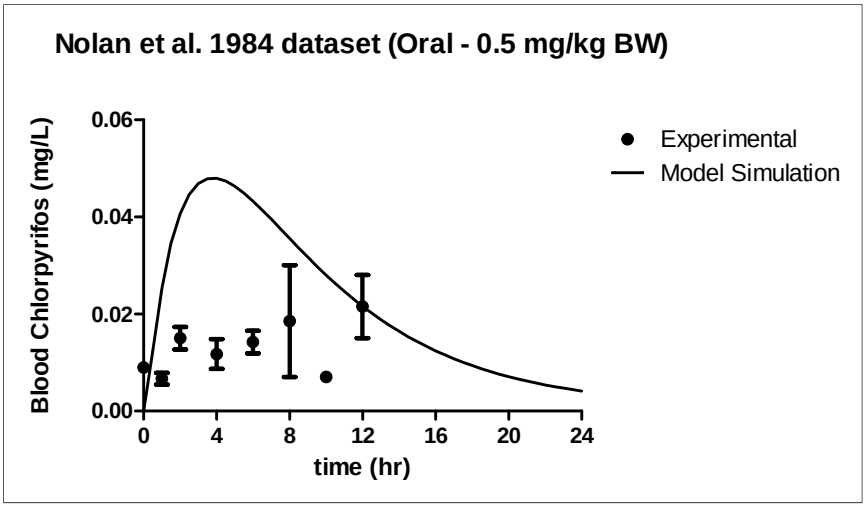

Figure 1A: Predicted human venous blood levels (mg/L) of chlorpyrifos from a single oral dose of 0.5 mg/kg BW chlorpyrifos against observations from the Nolan 1984 study.

The second simulation was the time course of plasma concentrations of TCPy after a single oral exposure to 0.5 mg chlorpyrifos/kg BW. Chlorpyrifos is primarily metabolized to TCPy either directly or following conversion to chlorpyrifos oxon. Plasma concentrations of TCPy represent a critical dose metric to evaluate because the TCPy-specific parameters were estimated by fitting model simulations to the TCPy data from the Nolan study. It was found that the model predictions and the experimental observations are in excellent agreement (Figure 2A). This finding increases our confidence in that the TCPy-specific parameters were adequately estimated.

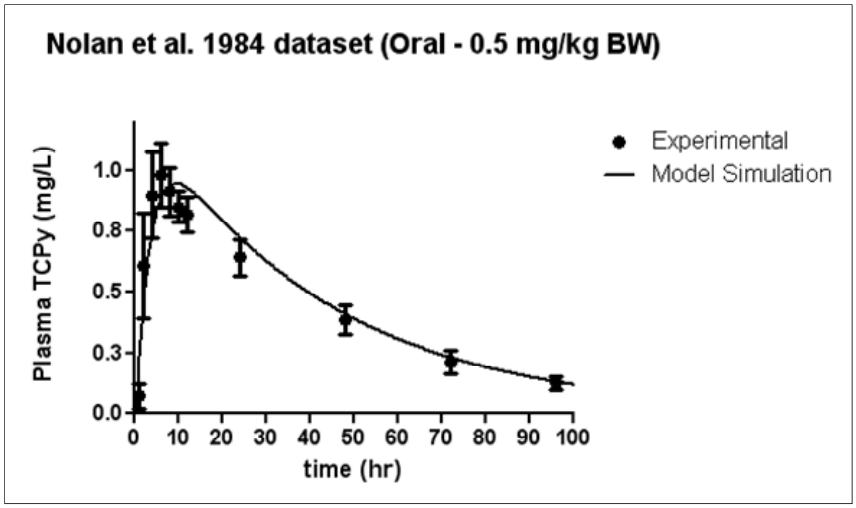

Figure 2A: Predicted plasma TCPy levels resulting from a single oral dose of 0.5 mg/kg BW chlorpyrifos based on the Nolan 1984 study

The third simulation was the time course of urinary excretion rate of TCPy in ug/hr as originally reported in the DER. Simulations reported by DAS and others have always been based on cumulative amount excreted, which requires additional manipulation of the original data (summation for a period of time to give cumulative amount excreted). It was found that the model predictions agreed well with the data, except the model under-predicted the peak urinary excretion rate of TCPy at 12 hours (Figure 3A).

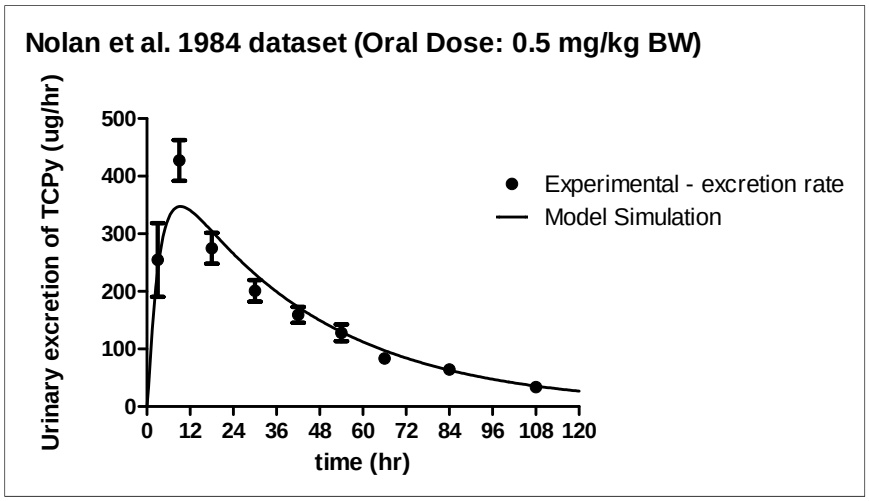

Figure 3A: Predicted urinary excretion rate resulting from a single oral dose of 0.5 mg/kg BW chlorpyrifos based on the Nolan 1984 study

(b). Nolan Dermal Study

Human volunteers in the Nolan Study were also exposed dermally with a 5 mg/kg BW dermal dose of chlorpyrifos 30 days after the oral dose. The dermal dose was administered by spreading ~10 µL/kg chlorpyrifos over ~100 cm^2 surface of the volar forearm. The first simulation was the time course of plasma concentrations of TCPy after a single dermal exposure to 5 mg chlorpyrifos/kg BW. The model predictions were in good agreement with the data reported in the DER (Figure 1B). The second simulation was the time course of urinary excretion rates of TCPy in units of ug/hr as originally reported in the DER. The model predictions were also in good agreement with the data (Figure 2B).

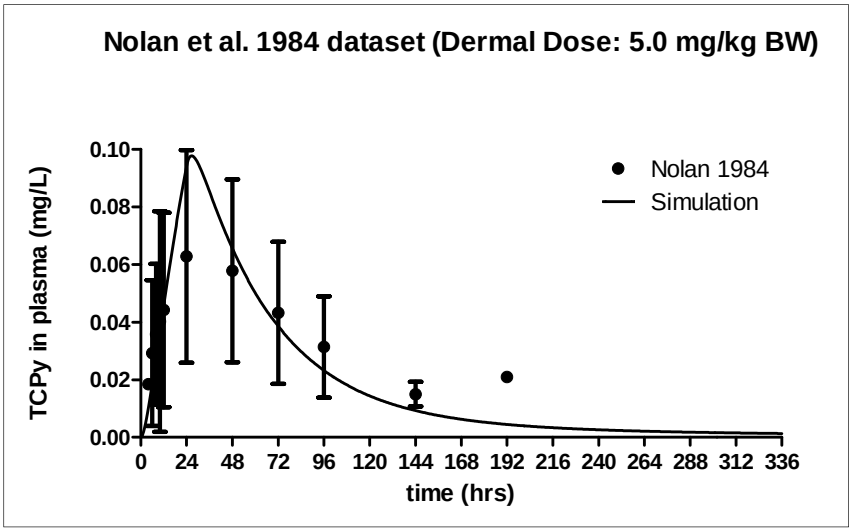

Figure 1B: Predicted plasma levels of TCPy resulting from a single dermal dose of 5.0 mg/kg BW Chlorpyrifos based on the Nolan 1984 study.

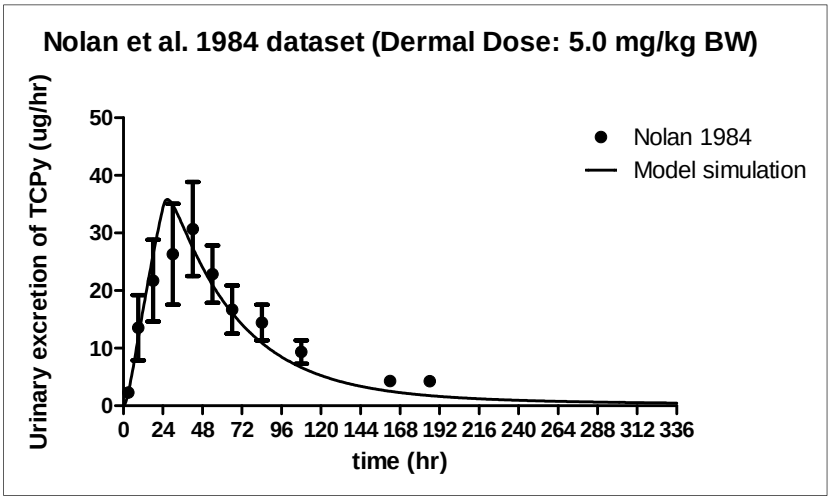

Figure 2B: Predicted urinary excretion rate of TCPy resulting from a single dermal dose of 5.0 mg/kg BW chlorpyrifos based on the Nolan 1984 study

In addition to TCPy in plasma and urine, the PBPK-PD model was used to simulate the time course of RBC and brain AChE inhibition associated with the single dermal dose of 5 mg/kg BW. The results showed a 13% brain AChE inhibition and a 1.5% RBC AChE inhibition (Figures 3B and 4B); these results did not reflect what is known about cholinesterase inhibition resulting from exposure to chlorpyrifos: plasma and RBC cholinesterase inhibition should precede any brain AChE inhibition. After communicating with DAS about this finding, they concur that the dermal component of the model is not recommended at this time to be used to evaluate brain metrics associated with dermal exposures to chlorpyrifos. However, the model can be used to evaluate other relevant cholinesterase inhibition metrics (e.g., plasma cholinesterase and RBC AChE inhibition).

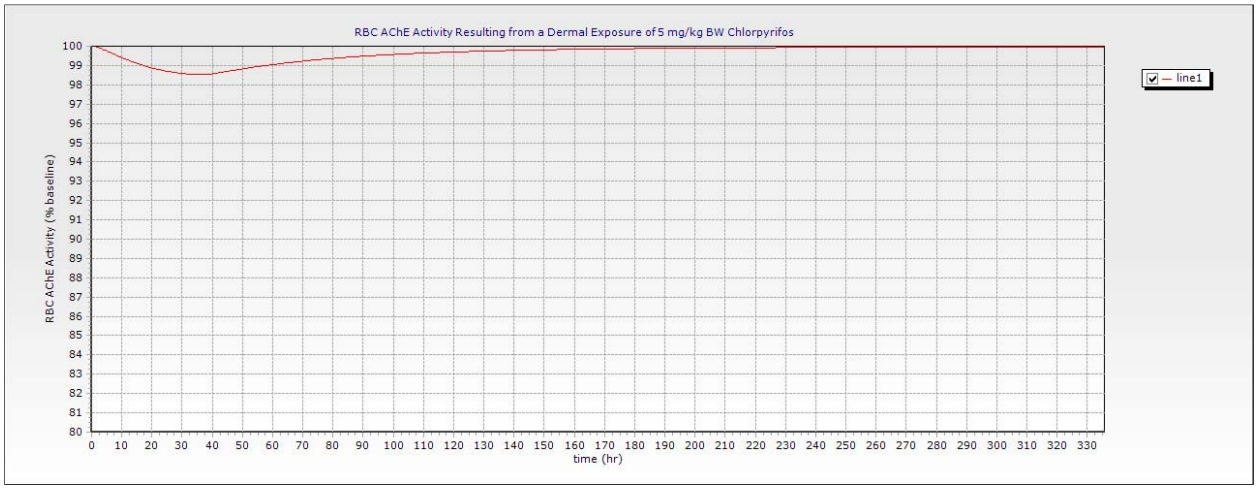

Figure 3B: Predicted RBC AChE inhibition resulting from a dermal exposure of 5 mg/kg BW chlorpyrifos based on the Nolan 1984 study.

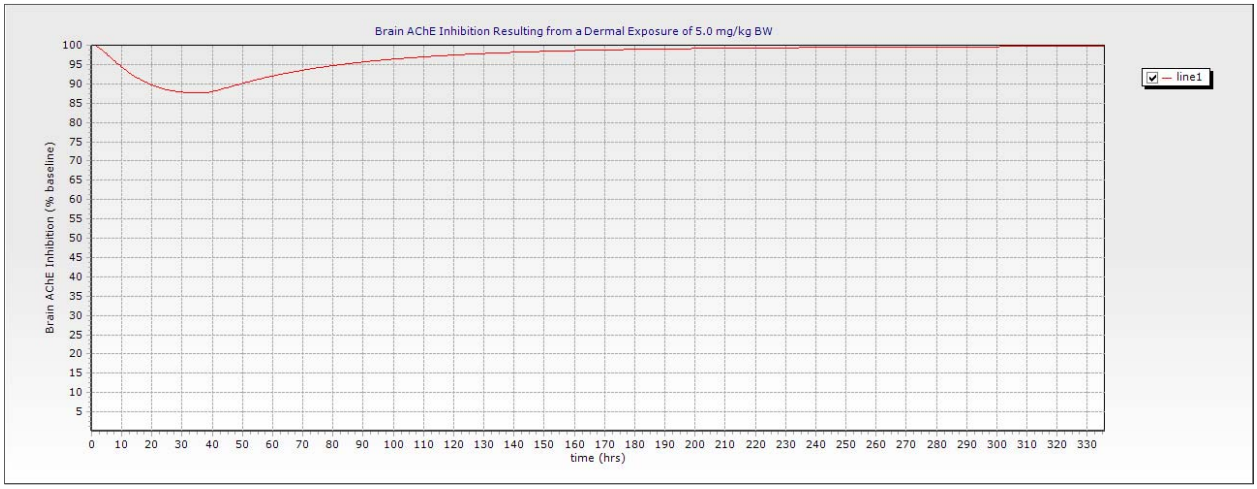

Figure 4B: Predicted brain AChE inhibition resulting from a dermal exposure of 5 mg/kg BW chlorpyrifos based on the Nolan 1984 study.

(c). Kisicki 1999 Oral Study

The PBPK-PD model calibrated using the Nolan Study was independently evaluated with data collected in the second human study in which 6 male and 6 female volunteers were administered 0.5, 1 or 2 mg chlorpyrifos/kg BW in a capsule (Kisicki *et al.*, 1999). While most model parameters remained unchanged, oral absorption rate was adjusted due to difference in the administered formulation containing chlorpyrifos in the two studies. Thus, evaluating the model using the Kisicki data was not completely independent as it should be. Yet, this exercise can still gauge the capability of the model in predicting internal dosimetry at different doses. The model was used to simulate the time course of TCPy concentrations in plasma and RBC AChE activities at three oral doses. It was found that the model under-predicted TCPy concentrations in plasma by a factor of two (Figures 1C, 2C, 3C), but the model was able to predict RBC AChE activities reasonably well (Figures 4C, 5C, 6C). Because AChE inhibition reflects the bioactivation of chlorpyrifos that results in the toxicity endpoint of concern (i.e., RBC AChE inhibition), the model performance is considered adequate for risk assessment application.

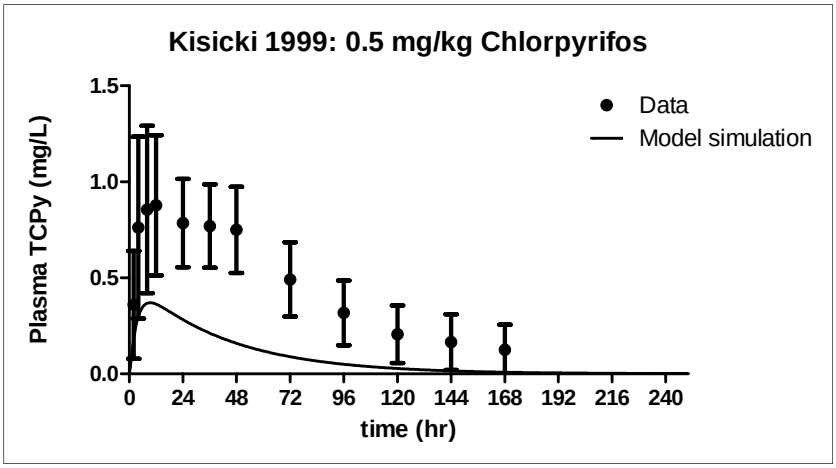

Figure 1C: Predicted plasma levels of TCPy resulting from a single oral dose of 0.5 mg/kg Chlorpyrifos

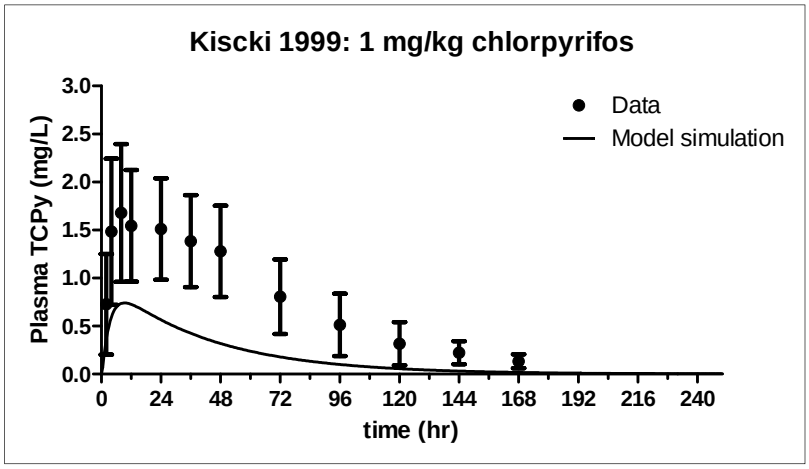

Figure 2C: Predicted plasma levels of TCPy resulting from a single oral dose of 1 mg/kg chlorpyrifos according to the Kisicki 1999 study.

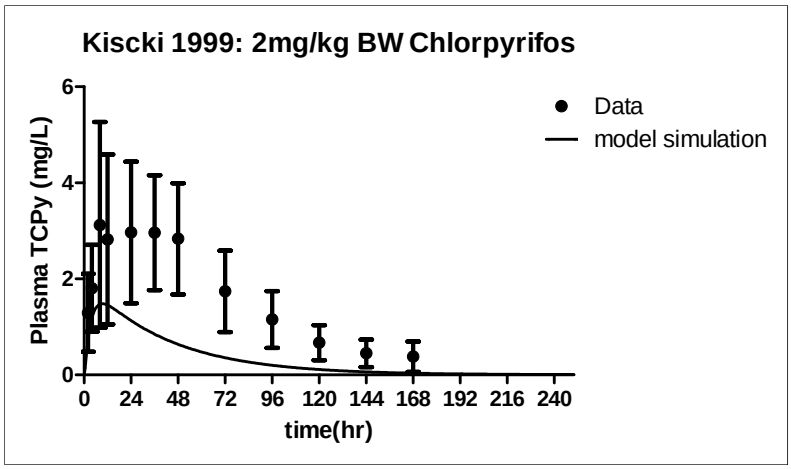

Figure 3C: Predicted plasma levels of TCPy resulting from a single oral dose of 1 mg/kg chlorpyrifos according to the Kisicki 1999 study.

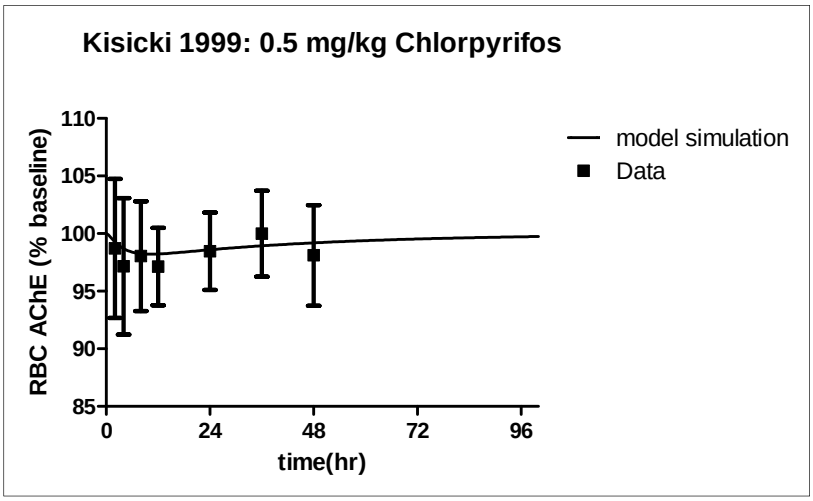

Figure 4C: Predicted % RBC AChE activity following an oral dose of 0.5 mg/kg BW chlorpyrifos according to the Kisicki 1999 study.

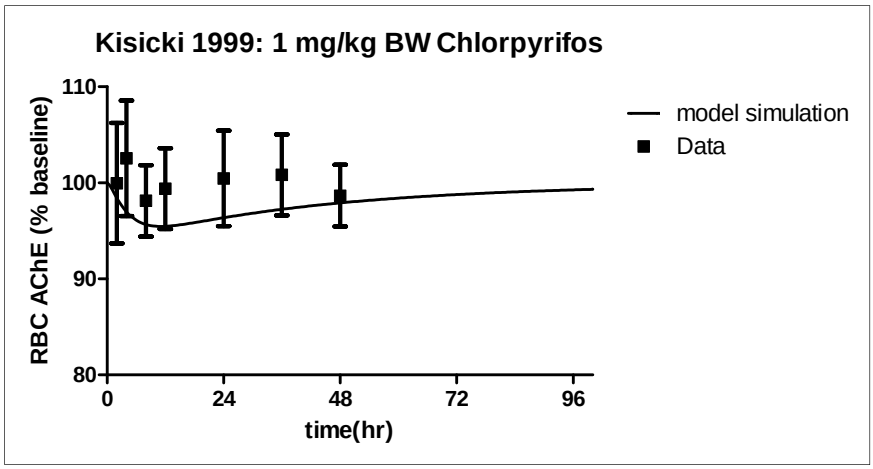

Figure 5C: Predicted % RBC AChE activity following an oral dose of 1.0 mg/kg BW chlorpyrifos according to the Kisicki 1999 study

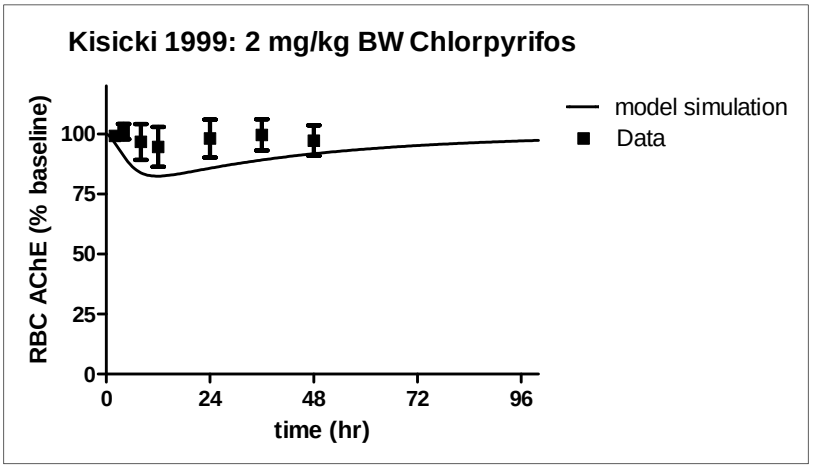

Figure 6C: Predicted % RBC AChE activity following an oral dose of 2 mg/kg BW chlorpyrifos according to the Kisicki 1999 study.

(V). Variability and uncertainty analysis

DAS recently estimated chemical-specific adjustment factors (CSAFs) by evaluating the variation and uncertainty in the ability of both chlorpyrifos and oxon to cause AChE inhibition in RBC in humans from oral studies. These variation and uncertainty were estimated using the chlorpyrifos PBPK-PD model to incorporate age-related differences in physiology, behavior, metabolism, and cholinesterase inhibition. In their approach, only four parameters were allowed to have age-dependent variations and uncertainty, while the other 124 parameters were set to fixed values. These four parameters were CYP450 activation of chlorpyrifos to oxon (VMHHCO); CYP450 detoxification of chlorpyrifos to TCPy (VMHCP); PON1 detoxification of oxon to TCPy in liver (VML); and PON1 detoxification of oxon to TCPy in plasma (VMBL1), and they were selected based on the results of following four steps:

1. Local sensitivity analyses by which parameter values are individually varied were conducted to determine the parameters that significantly affect RBC AChE;
2. Parameters identified in Step 1 were further assessed to determine whether they vary across a population;
3. Two global sensitivity analysis tests, the Morris screen test and the extended Fourier Amplitude Sensitivity Test (eFAST), were conducted to determine the parameters that significantly affect RBC AChE; and
4. Multiple Monte Carlo analyses were used to determine if a subset of the parameters identified in Steps 2 and 3.

Both local and global sensitivity analyses (Steps 1 and 3) are commonly used methodology to identify sensitive parameters in a PBPK model, and estimating the variation of model outputs by varying only sensitive parameters is also a common practice in the field. Approaches taken in Steps 2 and 4, however, identified some parameters for additional consideration.

Twenty seven parameters were identified as sensitive parameters in Step 1; and 12 of the 27 parameters are related to enzyme-mediated reactions (V_{max} and K_m). At low dose, variation in enzyme activity can be characterized by V_{max}/K_m, thus, only one of the two parameters can be set to constant. We agreed with the decision of not varying six sensitive K_m values. In addition to these metabolism-related parameters, seven other parameters were decided to set to fixed values. These parameters were plasma protein binding of oxon (FBO), plasma protein binding of chlorpyrifos (FBC), liver:blood partition coefficient for chlorpyrifos (PHO), coefficient for age-related changes in blood volume in average individual (VBL0), RBC degradation rate (KDRBCE), RBC AChE aging rate (KARBCE), and turnover rate for carboxyl esterases (TRCR). No evidence was provided in the November 2013 report provided by DAS to support the assumption that these parameters do not vary across individuals. Upon requesting, DAS provided additional justification for excluding the "non-varying" parameters. In addition, the registrant re-ran the local sensitivity analysis with two modified parameters: free fraction of chlorpyrifos (FUBC = 1-FBC), and free fraction of oxon (FUBO = 1-FBO). Such change was appropriate since the free fraction is the available fraction to be metabolized and partitioned into tissues.

The additional local sensitivity analysis conducted with FUBC and FUBO, instead of FBC and FBO, showed that FUBC was not a sensitive parameter, but FUBO still was a sensitive parameter. While FUBO was a sensitive parameter, it was excluded from the final Monte Carlo

simulations because dose-response analysis has shown that the effect of oxon binding to plasma protein has no significant impact on RBC cholinesterase inhibition below the dose level of 0.3 mg/kg.

Also, during the re-running, DAS noticed that they had incorrectly varied the liver volume as a function of age (VH0) in their first local sensitivity analysis since this parameter is an estimate of the central tendency of liver volume for individuals at a specific age, and not a measure an individual's characteristics or of variation in the population. Thus, VH0 was excluded from the final Monte Carlo simulation. While these changes and errors do not have a significant impact on the final result, they should have been identified by the QA conducted by the Summit Toxicology, giving us less confidence on their QA assessment.

DAS also provided clarification on their Step 4 in which the 14 sensitive parameters identified in Step 3 were further reduced to only four parameters that were varied in the final model. In general, the main reason for minimizing the number of parameters to vary in a Monte Carlo simulation is to conserve computational resources. In their Step 4, Monte Carlo simulations were run for "full model" (i.e., varying all 14 parameters) and several "smaller models" (i.e., varying subsets of 14 parameters), suggesting that computational resources was not limited. Therefore, we requested clarification for not including all 14 parameters in the final model. DAS's response was "by focusing the smaller number of varied parameters issues such as uncertainty in the mean and coefficient of variance of the distributions of interindividual variation in the parameters and correlations between parameters can be directed to the critical parameters", which was considered reasonable. DAS further showed that if all 14 parameters were varied, the estimated interspecies CASF will remain the same, but the intraspecies CASF would increase a small degree from 2.8 to 3.1 for chlorpyrifos.

(VI). Summary and Conclusion

This QA assessment identified limitations of the model capability and minor flaws in model documentation. It also achieved clarification and minor correction in the inter-individual variability analysis performed using the model. The major limitation of the current chlorpyrifos PBPK-PD model is that it lacks the ability to reasonably predict brain AChE inhibition resulting from dermal exposures. This limitation, however, is not considered a major setback for using the model in human health risk assessment because the model is still able to reasonably simulation RBC AChE inhibition, which is the metric of interest for risk assessment. The main flaw in model documentation identified in the QA assessment was the misidentification of sources of two model parameters, fraction of plasma protein bounded chlorpyrifos and chlorpyrifos-oxon. This issue has been documented in the model files. Further, clarification has been provided with additional references and simulations to justify the process of identifying critical model parameters that were included in the inter-individual variability analysis.

VII. References

Timchalk C, Nolan RJ, Mendrala AL, Dittenber DA, Brzak KA, Mattsson JL. A physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model for the organophosphate insecticide chlorpyrifos in rats and humans. Toxicol Sci 2002; 66:34–53.

FIFRA Scientific Advisory Panel. (2011). "Chlorpyrifos Physiologically Based Pharmacokinetic and Pharmacodynamic (PBPK-PD) Modeling linked to Cumulative and Aggregate Risk Evaluation System (CARES)." Report from the FIFRA Scientific Advisory Panel Meeting of February 15-18, 2011. FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. Available: http://www.epa.gov/scipoly/sap/meetings/2011/index.html.

FIFRA Scientific Advisory Panel. (2012). "Scientific Issues Concerning Health Effects of Chlorpyrifos" Report from the FIFRA Scientific Advisory Panel Meeting of April 10-13, 2012. FIFRA Scientific Advisory Panel, Office of Science Coordination and Policy, Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. Washington, DC. Available: http://www.epa.gov/scipoly/sap/meetings/2011/index.html.